

TABLE 1. MAA immunophenotyping of melanoma vaccine

Antisera	MAA defined (kilodaltons)	Presence of MAA (vaccine batch)			Ref.
		1	2	3	
Mouse monoclonal					
225.28S	240 +	+	+	+	23
9.2.27	240 +	+	+	+	24
436.G10	122-130	0	NT <sup>a</sup>	NT	25
Nu4B	26, 29, 95, 116	+	NT	NT	17
376.96	94	0	NT	NT	15
118.1	94-97	+	+	+	27
465.12S	94	0	NT	NT	26
McTBT	69-70	0	NT	NT	
Rabbit polyclonal					
SB29, SB54	240	+	+	+	11
SB29, SB54	150 <sup>a</sup>	+	+	+	11
SB29, SB54	140 <sup>a</sup>	+	+	+	11
SB29, SB54	120	+	+	+	11
SB29, SB54	95	+	+	+	11
SB29, SB54	75	+	+	+	11

<sup>a</sup> Not reactive with SB54.

<sup>b</sup> (NT) not tested.

TABLE 2. *Effect of detergent and ultracentrifugation on macromolecules, MAAs, and Dr antigens in material shed by melanoma cells*

Treatment <sup>a</sup>	Presence in shed material after ultracentrifugation					
	<sup>125</sup> I-macromolecules <sup>b</sup>		<sup>125</sup> I-MAAs <sup>c</sup>		<sup>125</sup> I-Dr <sup>c</sup>	
	Change <sup>d</sup>		Change		Change	
	cpm	(%)	cpm	(%)	cpm	(%)
None	10,362		817		428	
Ultracentrifugation	6,325	-40	258	-70	0	-100
NP-40 + ultracentrifugation	8,612	-17	574	-30	0	-100

<sup>a</sup> Material shed by radioiodinated melanoma cells was ultracentrifuged in 0.5-ml aliquots at 100,000 g for 90 min, incubated in a final concentration of 0.5% NP-40 for 2 h prior to ultracentrifugation, or not treated. All were subsequently assayed for radioactivity associated with macromolecules, MAAs defined by antiserum SB29, or Dr antigens. All assays were performed on 0.025-ml aliquots of material in the presence of a final concentration of 0.5% NP-40.

<sup>b</sup> Assayed by precipitation with 10% trichloroacetic acid.

<sup>c</sup> Assayed by protein A-immune precipitation with specific antisera.

<sup>d</sup> From untreated control.

TABLE 3. Surface MAAs expressed by melanomas in various individuals

MAA	Antiserum	Expression of MAA in melanoma*												
		HM31	HM34	HM49	HM54	HM60	HMR0	GJ61	SK23	SK27	SK28	SK29	SK37	VAL
240 +	SB29	+	+	-	+	+	-	+	+	+	+	+	+	+
	SB34	+	+	-	+	+	-	+	+	+	+	+	+	+
	225.28S	-	-	-	+	+	+	-	+	+	+	+	+	-
	9.2.27	-	-	-	+	+	+	-	+	+	+	+	+	-
150	SB29	+	+	+	+	+	-	+	+	+	+	+	+	-
140	SB29	+	+	+	+	+	-	+	+	+	+	+	+	-
120	SB29	+	+	+	+	+	-	+	+	+	+	+	+	-
	SB34	+	+	+	+	+	-	+	+	+	+	+	+	-
116	Nu4B	-	-	-	-	-	-	-	-	-	-	-	-	+
95-97	SB29	+	+	-	+	+	-	+	+	+	+	+	+	+
	SB34	+	+	-	+	+	-	+	+	+	+	+	+	+
	11R.1	-	-	-	+	+	+	+	+	+	+	+	+	+
75	SB29	+	+	-	+	+	+	+	+	+	+	+	+	+
	SB34	+	+	-	+	+	+	+	+	+	+	+	+	+
70	Me3 TBT	-	-	-	-	-	-	-	-	-	-	-	-	-

\* Assayed by indirect immunoprecipitation with protein A-sepharose.

TABLE 4. *Characteristics of immunized patients*

Patient no.	Age	Sex	Previous treatment other than surgery	Site of metastasis	Duration of metastatic disease prior to immunization (months)	No. of immunization	Current status <sup>a</sup>	Length of follow-up <sup>b</sup> (months)
1	31	F	BCG, <sup>c</sup> DTIC	Skin, lung	12	10	P	1/4
2	24	F	None	Lung	2	10	P	3
3	53	M	None	Skin	2	13		8
4	58	F	None	Skin	2	8	P	1
5	48	M	None	Skin	1	12	P	3
6	54	M	None	Skin	2	11	P	3
7	58	M	None	Skin	2	14	P	4
8	68	M	None	Skin, lung	2	10	P	6
9	75	F	DTIC, Actinomycin D	Skin	36	17	S	14
10	46	M	None	Skin	1	18	R	24
15	29	M	None	Skin	4	8	P	2
17	68	M	None	Skin	4	8	P	2
20	38	M	None	Skin, lung	3	13	P	4

<sup>a</sup> (P) progression; (S) stable; (R) regression.

<sup>b</sup> From onset of immunotherapy.

<sup>c</sup> (BCG) bacillus Calmette-Guérin.

TABLE 5. Immunogenicity of melanoma vaccine

Patient no.	Immune response to melanoma <sup>a</sup>		
	Humoral <sup>b</sup>	Cellular <sup>c</sup>	Either
1	++	0	5
2	+	10	+
3	+	0	+
4	0	0	0
5	±	5	0
6	0	0	0
7	0	0	+
8	0	0	0
9	0	20	+
10	+	10	+
15	++	0	+
17	0	NT	0
20	NT	25	+
No. (%) positive:	5 (38%)	4 (31%)	8 (62%)

<sup>a</sup> (±) 25–49%, ( + ) 50–100%, and ( + + ) 100%, increase over preimmunization level of melanoma antibodies; (NT) not tested.

<sup>b</sup> By indirect immunoprecipitation of <sup>125</sup>I-melanoma macromolecules.

<sup>c</sup> By skin test to 10 µg vaccine; results represent millimeters of average induration at 24–48 h.

**TABLE 6. Antibodies to fetal calf serum proteins in patients immunized to melanoma vaccine**

Patient no.	Antibodies to preimmunization	<sup>125</sup> I-FCS* (2 months' postimmunization)
<b>Melanoma</b>		
1	18.3	0.7
2	0.0	0.1
3	0.0	0.0
4	0.1	0.1
5	0.1	0.2
6	0.1	<0.1
7	<0.1	0.1
8	<0.1	<0.1
9	0.6	0.8
10	0.0	0.0
15	0.1	<0.1
17	0.3	0.4
20	0.0	0.0
<b>Normal</b>		
2003	0.0	
2004	0.1	
2005	<0.1	
2006	0.0	
2007	0.0	
2008	0.0	
2009	0.0	
2010	0.1	
2011	0.0	
2012	0.0	
2013	0.0	
<b>ANTI-FCS</b>	<b>68.0</b>	

\* Percent of radioactivity associated with <sup>125</sup>I-FCS specifically immunoprecipitated by serum and protein A-sepharose.